FINAL REPORT

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CANOLA-QUALITY OILSEED SINAPIS ALBA WILL BE DEVELOPED INTO AN OILSEED CROP FOR THE DRYLAND PRAIRIE

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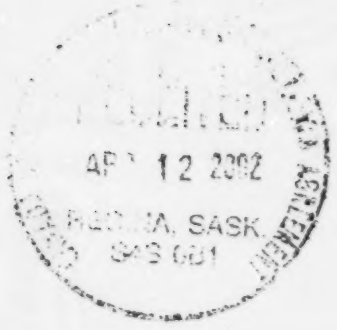

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September 1, 1997 to March 31, 2002

SUMMARY

The development of *Sinapis alba* into a vegetable oilseed crop presents tremendous economic, agronomic, and environmental potential benefits for stakeholders (e.g. growers, processors, marketers) in the Canadian vegetable oilseed industry. Through this research, Canada has a unique opportunity to lead the world in developing a new, high value crop, and to subsequently enhance its competitiveness as a reliable producer of high quality vegetable oilseed. Oilseed *S .alba* has the potential to expand Canada's production of vegetable oilseed crops into the semi-arid prairies of Alberta and Saskatchewan, where current canola species (*Brassica napus* & *Brassica rapa*) do not thrive. Unlike canola, *S. alba* is adapted to the hot, dry conditions of the semi-arid Prairies, where it is already successfully produced as a condiment mustard. *Sinapis alba* has several important agronomic advantages over *Brassica* species, including large seed size, rapid establishment, early maturity and resistance to shattering. It has resistance to important diseases (e.g. blackleg, and *Alternaria)* and insect pests (e.g. flea beetles, diamond back moth and cabbage seedpod weevil). Sclerotinia stem rot is not usually a problem because *S. alba* is grown in drier conditions which do not favor disease development.

Oilseed *S. alba* is strategically being positioned as a non-GMO crop, and as an option for organic production. No recombinant DNA technology is employed or planned in the development of oilseed *S. alba*. Furthermore, there is no expectation of contamination from canola GMO's since there are strong biological barriers which prevent cross fertilization between *S. alba* and *Brassica* species. Organic production of oilseed *S. alba* is possible because rapid stand establishment makes *S. alba* highly competitive with weeds and reduces the need to apply herbicides. In addition the strong disease and pest resistance profile of *S. alba* makes the crop an ideal candidate for organic production.

The production of oilseed *S. alba* in the semi-arid prairies has an estimated value of $2,980 million annually from the marketing of vegetable oil and an additional $992 million from the marketing of high protein meal. Market demand for oilseeds is increasing steadily, and in order for Canada to effectively increase its market share, vegetable oilseed production must increase significantly, beyond the capacity of the current *Brassica* canola species.

This AFIF research focused on developing vegetable oilseed *S. alba* from condiment *S. alba,* the first requirement of which was to reduce the content of erucic acid and glucosinolates in the seed. Strains of *S. alba* were developed possessing <0.1% erucic acid in the oil as well as <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate in the meal. This result was critical to the future success of oilseed *S. alba* as an edible vegetable oilseed crop, since only a minimal content of erucic acid and glucosinolates are permitted in a vegetable oilseed crop. The second major result of this project was the development of a broad based oilseed *S. alba* population. The desirable traits of <0.1% erucic acid content, <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate were incorporated into a wider genetic background. This population has also been introgressed with genes for high oil content, high protein content and good agronomic adaptation. Subsequent selection within this population is expected to result in a) high yielding, high-quality strains of oilseed *Sinapis alba* for the vegetable oil market; and b) high yielding, high protein content, strains of *S. alba* as a source of high quality animal feed and possibly for human consumption. With the development of a broad based oilseed *S. alba* population, this research has advanced beyond the proof of concept stage.

Field evaluation of oilseed *S. alba* lines indicated an increase in oil content of 3.5% as a result of three back crosses to a high oil content breeding line. Similarly recurrent selection was effective in increasing protein content by 3% following three cycles of selection in a condiment yellow mustard population. A high protein content line from this improved population was used as parent in developing the broad based oilseed *S. alba* population.

This research generated valuable information on the inheritance of erucic acid and hydroxybenzyl glucosinolate. Erucic acid content in *S. alba* is determined by the genotype of the embryo, and controlled by a single gene, exhibiting partial dominance of high over low erucic acid. Hydroxybenzyl glucosinolate is also a single gene trait. It is under maternal control, with dominance of high over low glucosinolate content. Molecular markers for erucic acid were also developed.

Multi-location germplasm evaluation trials also provided information on the variability that is present in *S. alba* germplasm accessions and the usefulness of such accessions for subsequent breeding efforts. Germplasm accessions provide a different source of variation and will benefit both the oilseed and condiment *S. alba* breeding programs. Screening *S. alba* germplasm for white rust and blackleg reaction demonstrated that *S. alba* is resistant to these diseases, and breeding for oilseed quality traits (reducing erucic acid and glucosinolate content) has not resulted in the introduction of novel vulnerabilities with respect to these diseases.

The germplasm and information generated in this research has laid a strong foundation for the future development of oilseed *S. alba* as a crop in western Canada.

TECHNICAL REPORT

Background and Objectives

Canada is a world leader in the vegetable oilseed industry, and canola is an important crop for Canadian prairie farmers, averaging 4.24 million tonnes on 3.3 million ha over the 10 year period from 1985-1994 (Anonymous, 1995). World market demand for high quality vegetable oil is increasing, and for Canada to participate in this expanding market, and fortify its position as a world leader, oilseed production must increase significantly. The currently cultivated species of canola *Brassica napus* and *Brassica rapa* will not be able to meet anticipated increase in demand, because optimum canola production is limited to regions with cool temperatures and low evaporation rates; such as those typically found in the canola belt.

Sinapis alba (yellow mustard) has the potential to be developed as an oilseed crop in western Canada. It is well adapted to the Brown and Dark Brown soils of the Prairies and Great Plains and grows well in the semi-arid growing conditions of the southern Prairies of western Canada. The crop is more tolerant to heat and drought than canola species (*B. napus* & *B. rapa)* and is highly shatter resistant (Rakow, 1997). The seed of *S. alba* is bright yellow, large (5.5 to 6.5 g/1000 seed) and, in the case of a dry spring, can be deep seeded (50 mm) into more moist soil (Brandt, 1992). Seedling emergence and stand establishment is vigorous and rapid, making *S. alba* highly competitive with weeds. The seedlings respond to drought stress by rapidly increasing root surface area with a proliferation of small fibrous roots. The root system in *S. alba* is deeper and grows more rapidly in drought conditions than canola roots, giving yellow mustard accessibility to deeper soil moisture (Vartanian, 1973). Yellow mustard also escapes summer drought by its rapid growth, so that the most heat sensitive developmental periods of flowering and seed set are well established before the onset of mid-summer heat and drought in the semi-arid prairies. *Sinapis alba* grows to a height of 95 to 103 cm, is early maturing (91 days), and does not have problems with lodging, shattering, green seed and chlorophyll.

Sinapis alba is resistant to blackleg (*Leptosphaeria maculans* or *Phoma lingam*) (Weber *et al.*, 1996) and *Altenaria* blackspot (Brun *et al.*, 1989; Hausen *et al.*, 1995; Zhu and Spanier, 1991), two important diseases of canola in western Canada (Gugel and Petrie, 1992). This species also exhibits high levels resistance to flea beetles (*Phyllotreta cruciferae*) (Bodnaryk and Lamb, 1991; Brandt and Lamb, 1994; Lamb, 1984; Palanisawamy and Lamb, 1992; Palanisawamy *et al.*, 1997; Putnam, 1977). The basis of this resistance has been demonstrated to be antixenosis and good tolerance to feeding damage. Yellow mustard exhibits more rapid compensatory growth following flea beetle damage, relative to *B. juncea, B. rapa* and *B. napus* (Bodnaryk, 1996; 1997; Bodnaryk and Lamb, 1991; Brandt and Lamb, 1994). Lamb (1980) also concluded that hairs protected pods of *S. alba* from flea beetle damage. Levels of resistance to white rust (*Albugo candida*) are moderate. *Sinapis alba* has good tolerance to diamond back moth *(Plutella xylostella)* (Brown *et al.*, 1996; Reed *et al.*, 1989), root maggot (Dosdall *et al.*, 1994) and also exhibited some resistance to *Rhizoctonia solani* pre-emergence damping off and post emergence seedling root rot (Yang and Verma, 1992; Yang *et al,* 1992). The good disease and pest resistance profile

coupled with an aggressive competitive seedling growth habit make *S. alba* a good candidate crop for organic production, free of fungicides, pesticides and herbicide applications. In addition, *S. alba* does not intercross with *Brassica* species and this makes the species a good candidate for the development of an oilseed that is free of genetically modified events (GMO-Free).

High oil content oilseed *Sinapis alba*

Oils from *Brassica* seed crops are characterised by high levels of long-chain monoenoic fatty acids, eicosenoic and erucic acid (Downey and Rakow, 1987). The erucic acid content in *S. alba* seed oil is 25-35%. The predominant glucosinolate in *S. alba* is hydroxybenzyl glucosinolate, which is present at rates of 120-180 μmoles g^{-1} seed in western Canadian condiment mustard cultivars. There are compelling health and nutritional justifications for reducing the contents of erucic acid in vegetable oil and glucosinolates in the meal. Although erucic acid toxicity has not been demonstrated in humans, this acid has been associated with poor performance and abnormal lipid metabolism in heart and skeletal muscle tissue in laboratory animals and pigs (Abdellatif *et al.*, 1970; Holmes, 1980; Laryea *et al.*, 1992). The evidence correlating glucosinolates to poor animal performance as well as to poor human health is strong (Bell, 1977; Bowland *et al.*, 1965; Fenwick *et al.*, 1983) Glucosinolates reduce animal palatability and feed intake in non-ruminant animals, and inhibit iodine uptake by the thyroid gland, causing goitre and other growth and reproductive anomalies. The reduction of erucic acid and glucosinolates through breeding has been successfully employed in *Brassica* to develop canola (Downey and Rakow, 1987).

With the identification of low erucic acid *S. alba* strains at the Saskatoon Research Centre in the 1970's, and the development of low glucosinolate strains in Poland (Krzymanski *et al.*, 1991), it is now possible to develop good-quality edible oilseed forms of *S. alba* (Raney *et al.*, 1995). The fatty acid profile of edible oilseed *S. alba* lines is excellent, with potential to produce oil containing both high oleic acid (>73%) and low linolenic acid (<4%). The development of edible oilseed *S. alba* will extend the oilseed growing area south to the drier regions of Saskatchewan and Alberta where current canola production is low. *Sinapis alba* oilseed will provide growers in the southern prairies with an alternative to cereal production, and will benefit the canola industry as a whole by increasing and stabilizing the supply of good quality vegetable oilseed.

High Protein *Sinapis alba*

Oilseed *S. alba* will have significant value as a high protein supplement. The international market for animal feed rations is large, valued at $2.7 billion, of which oilseed meal is approximately 13 % (Mupondwa, 1999). This market is expected to grow as animal protein consumption continues to increase around the world. In Canada, there is a shortage of locally produced protein supplement, therefore much of the local protein supplement requirement is met with imported soybean meal. The quality of protein from canola meal is equal to that from soybean meal (Sarwar *et al.*, 1981). Seed meal from canola, however, is not fully competitive with soybean on the market. Canola seed is small, and the meal has a lower protein and higher fibre content resulting in lower digestibility or metabolizable energy compared to soybean (Bell, 1993). For these reasons, rapeseed meal is valued at 65 - 70 % of soybean meal. *Sinapis alba* however, has a high protein content (48 % in the meal) which is comparable to soybean protein content. The value

of *S. alba* protein is similar to canola (Bell and Rakow, 1996; Applequist and Nair, 1977) and therefore similar to soybean. Bell *et al.* (1997) concluded that the quality of *S. alba* protein as indicated by its content of indispensable amino acids was similar to *Brassica* protein. Applequist and Nair (1977) came to a similar conclusion, however Sarwar *et al.* (1981) concluded that *S. alba* protein was of inferior quality to rapeseed, based on essential amino acid content. The quality of *S. alba* protein requires further clarification. Breeding will readily increase protein content in *S. alba* to equal or surpass the protein content in soybean (48%). Reported fibre levels for *S. alba* (9 - 12%) are lower than in rapeseed (12 - 18%), but greater than in soybean (6 %) (Sarwar *et al,* 1981. Therefore, as with canola, dehulling would improve digestibility of the meal (Vose, 1974). Anjou *et al.* (1977) also reported that yellow mustard seed had less fat and lignin content but more protein and carbohydrate than rapeseed. Bell and Rakow (1996) reported that the content of trypsin inhibitors in oil extracted *S. alba* meal were between values for *B. napus* (3.58, 3.61 units/g) and *B. juncea* (1.26, 1.39, 1.56) which are significantly below those reported for soybean meal. *Sinapis alba* had the lowest value of phytic acid content (2.49). Menegatti *et al.*, (1985) also isolated and characterized a trypsin inhibitor from *S. alba* seed. The bright yellow seed of *S. alba* is also attractive as it blends uniformly into feed. High protein, low fat *S. alba* also has potential application as full fat on farm feed (Slominski, Personal communication). *Sinapis alba* also has potential to supplant soybean as high protein supplement for human consumption. It can have versatile applications in human diets, as a protein additive in prepared meats, dairy products, sauces, pastes, soups and possibly as a new vegetable protein products. Non-food industrial applications are also possible. The technology developed from this research will reduce the need to import soybean meal supplement, and allow Canada to capture a larger share of the international market for high quality protein supplements. It is also an opportunity for market and crop diversification.

Impact of the Research

This research is designed to develop strains of canola-quality oilseed *S. alba* that are high yielding, resistant to disease, well adapted to the semi-arid growing conditions of the Canadian prairies, with a high oil content or a high protein content. These strains will form the basis for the breeding of a new oilseed species in the southern Prairies. The successful development of oilseed *S. alba* will have implications for farmers and processors in the oilseed, livestock feed, human food and non-food processing industries. The single largest impact to farmers will be the expansion of the potential canola growing belt in the Brown and Dark Brown soil zone. Increased canola production will contribute to stabilizing the supply of Canadian canola in local and international markets. Another benefit to producers is the diversification of their crop production systems by including a high value oilseed crop which will stabilize farm income. The low chemical input in *S. alba* production will have both economic and environmental benefits to producers. Crushers can expect high crush margins and economic returns from oilseed *S. alba* (Mupondwa, 1999). Demand for good quality protein supplements is increasing as market demand for animal protein increases. *Sinapis alba* meal is expected to be more competitive to soybean meal than current canola meal, because of its significantly higher protein content. This is especially significant for poultry and pig rations. There is also potential to export this crop and compete on the world market for high protein meal supplements. The food industry provides an opportunity for value-added processing of high protein *S. alba*, similar to soybean processed products. Approximately 80-90 % of edible soybean protein products is consumed

in meat products either as extenders or functional ingredients. Opportunities for non-food uses are extensive and include varied products such as diesel, hand creams, cleaners, industrial solvents, adhesives and textiles.

Project Objectives

The objectives of this research are to develop: a) high yielding, high oil content, high quality strains of oilseed *S. alba* for the vegetable oil market; and b) high yielding, high protein content, high quality strains of *S. alba,* as a source of high quality animal feed, and possibly for human consumption.

Project activities, achievements, papers written

This project was organized around the following research activities:

1. Developing edible, oilseed quality strains of *S. alba* which are free of erucic acid in the oil and of hydroxybenzyl and benzyl glucosinolates in the meal.
2. Increasing oil content in oilseed *S. alba*
3. Evaluating *S. alba* germplasm accessions.
4. Increasing protein content in *S. alba*.
5. Studying the inheritance of erucic acid and hydroxybenzyl glucosinolate in *S. alba*.
6. Screening *S. alba* germplasm accessions for resistance to white rust
7. Screening oilseed *S. alba* lines for resistance to blackleg.

1. *Developing edible, oilseed quality strains of S. alba which are free of erucic acid in the oil and of hydroxybenzyl and benzyl glucosinolates in the meal.*

Oilseed *S. alba* was developed by introgressing genes from three germplasm pools into one population: i) a low erucic acid line (BHL-926) developed at the Saskatoon Research Centre by Drs. D. Woods and K. Downey in the 1970's; ii) a low glucosinolate line developed in Poland (Kryzymanski *et al.* 1991); and iii) a high oil content (36-38 % oil) line developed in Sweden (Olsson, 1974). The initial cross to combine low glucosinolate and low erucic acid content was made, between a low glucosinolate plant, 92-6669 [zero hydroxybenzyl glucosinolate, low total alkenyl glucosinolate (7.65 μmoles g^{-1} seed), high erucic acid content] and a low erucic acid content line BHL-926 (less than or equal to 1 % erucic acid, high glucosinolate) (Raney *et al.,* 1995). F4 progeny of this cross (<4 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <1% erucic acid) were crossed with the high oil content line from Sweden. F5 half seed analysis indicated that variation for zero erucic acid was absent. Thus F1 lines of the cross (F4 x High oil line) were crossed with a high oleic, zero erucic acid selection from BHL-926. Selections were made among F2 plants for oilseed-quality plants with <1% erucic acid and <1 μmoles g^{-1} seed hydroxybenzyl glucosinolate. Selected F2 plants were crossed to the high oil line for a second cycle of gene introgression

for high oil content. F3 plants from this cross were evaluated for oil content in a replicated field trial, with two checks, the high oil line (39.5% oil) and AC Pennant (32% oil). Twelve lines with the highest oil content (35.9-36.8 %) were analysed for fatty acid profile, and plants with the highest oleic acid content (>73%) were open pollinated to produce F4 seed. Oil and protein content of the 43 OP F4 lines were evaluated in a replicated field trial in 1997. In 1998, Selections from these F4 lines were crossed with a low erucic acid content plant containing <0.1 μmoles g^{-1} seed benzyl glucosinolate (WD97-148), which was selected from the original cross between 92-6669 (low glucosinolate) and BHL-926 (low erucic acid).

Oilseed-quality lines which had <0.1% erucic acid content as well as <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate content, were evaluated in a replicated field trial in 1999 and 2000 to assess agronomic performance as well as oil and protein content. In the field, plants were grown in single 10 m rows, each with 100 plants. The rows were spaced 2 m apart and plots were arranged in a randomized complete block design with two replications and 101 entries. The Check entries were AC Base, a well adapted high yielding condiment variety developed at the Saskatoon Research Centre and Svalöf, a high oil content (36-38% seed oil) breeding line obtained from Sweden. Data was recorded on seedling emergence, stand establishment, vigor at different growth stages, flowering date, height and maturity. Seed samples were taken for chemical analyses (oil, protein, glucosinolates). Seed weight was determined as grams per 1000 seed, oil content was determined using Nuclear Magnetic Resonance (NMR) technique and protein content was determined using Dumas combustion method. Gas-liquid chromatography was utilized to analyze fatty acid (half seed or bulk seed basis) and glucosinolate content in the seed. Glucosinolate analysis was also made on floral buds.

The development of *S. alba* lines with both <0.1% erucic acid and <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate content was a major activity during the first two years of this project. However all the lines that possessed these desirable oil and meal quality traits, contained elevated levels of benzyl glucosinolate. This was consistent with the findings of Brown *et al.* (1997) who reported that as the levels of hydroxybenzyl glucosinolate decreased, levels of minor glucosinolate increased. At the Saskatoon Research Centre, elevated levels of benzyl, hydroxy-butenyl and indoly glucosinolates were detected in strains containing <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate. The introgression of genes for <0.1 μmoles g^{-1} seed benzyl glucosinolate into strains that contain <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate was undertaken effectively. Breeding efforts were initiated in 1997 to introduce the <0.1 μmoles g^{-1} seed benzyl trait into the oilseed-quality lines. Plants with <0.1% erucic acid and low benzyl glucosinolate content (2-5.4 μmoles g^{-1} seed) were crossed to a plant containing <0.1 μmoles g^{-1} seed benzyl glucosinolate. Selections for plants possessing <0.1 μmoles g^{-1} seed benzyl glucosinolate together with <0.1% erucic acid and <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate were made among F2 progenies of this cross.

In 1998, the major accomplishment was the development of oilseed-quality lines which combined the traits for <0.1% erucic acid, <0.1 μmoles g^{-1} seed hydroxybenzyl and <0.1 μmoles g^{-1} seed benzyl glucosinolate content (Tables 1& 2).

By 1999, the *S. alba* oilseed breeding program had successfully stabilized three important oil and meal quality traits; <1% erucic acid content, <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate content. These traits are all recessive and therefore occurred at a low frequency in a the breeding population, necessitating time and labour consuming activities in crossing and laboratory analysis. The fatty acid profile of the oilseed *S. alba* lines was excellent with potential to produce oil containing both high oleic acid (>73%) and low linolenic acid (<4%) (Table 1). In 1999 and 2000 *S. alba* lines possessing these high quality traits were evaluated in the field for agronomic performance.

field performance of high quality strains of Sinapis alba

In 1999 and 2000, one hundred and one high quality *S. alba* lines derived from three backcrosses with a European high oil line (Svalöf) and stabilized with respect to <0.1% erucic acid, <0.1 μmole g^{-1} seed hydroxybenzyl and benzyl glucosinolate were evaluated in a replicated field trial (two row plots and three replicates) at the Saskatoon Research Centre field station. The checks were AC Base (excellent agronomic adaptation) and Svalöf (high oil content). Establishment of these trials was good. Agronomic ratings of the experimental oilseed *S. alba* lines were, on average, less (3-4) than either check AC Base (5) or Svalöf (4). Days to flowering (DTF) of most lines were later than AC Base (40 DTF) reflecting the late flowering (46 DTF) Svalöf pedigree. (Table 3) The maturity of the oilseed lines was intermediate to that of AC Base (96) and Svalöf (101), reflecting the tendency for late maturity in the Svalöf parent. Similarly, the maturity of the oilseed lines was intermediate to that of AC Base (96) and Svalöf (101). Selection for earlier maturity (similar to AC Base) will therefore be an important goal in this material. Seed chemical and data analyses of oilseed *S. alba* indicated that this material was on average higher in oil content (32.4%) than AC Base (29.0%), and this was attributed to high oil content genes from Svalöf (37% oil). The range in oil content among the oilseed experimental lines was 31.9 - 34.1%.

Introgression of high quality traits into adapted high yielding lines of yellow mustard

Following the stabilization of the desired meal and oil quality traits, the next important activity related to expanding the germplasm base of oilseed-quality *S. alba*. This activity occurred from 1999 to 2001. Crosses were made in the greenhouse of selected high quality strains with a well adapted, high yielding, condiment, yellow mustard cultivar (AC Base) as well as with a well adapted, high protein content, experimental, condiment, yellow mustard line (SA97-751). These crosses were made specifically to begin the important process of introgressing desirable oil and meal quality traits (<0.1% erucic acid, <0.1 μmoles g^{-1} seed hydroxybenzyl and <0.1 μmoles g^{-1} seed benzyl glucosinolate content) into adapted genetic backgrounds. F1 plants were grown and selfed to produce F2 seed. Individual plants which possessed the traits for all three quality traits (<0.1% erucic acid, <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate content) were selected to be founder members of a population for recurrent selection. There is a low probability of individuals that possess all three traits concurrently, necessitated the screening of thousands of F2 plants to identify a sufficient number of plants with the desired combination of traits. These traits are all recessive, requiring the presence of a homozygous recessive condition at each locus. Selected F2 plants were intercrossed in the greenhouse, in 2000, and resultant seed was used to establish a field crossing block in 2001. This highly valuable crossing block required the maintenance of strict isolation distances from other yellow mustard in the field, especially condiment yellow

mustard. Further, the crossing block was grown in a field that was accessible to irrigation, to mitigate against loss due to severe drought. The irrigation system was used once, just prior to seeding.

The development of a broad based oilseed-quality population was an important milestone in the oilseed S. *alba* breeding program. This population constitutes the founder population for the new oilseed *S. alba* crop. The population has been introgressed with genes for high oil and low mucilage content from the breeding line Svalöf, as well as good agronomic performance from AC Base and high protein content from SA97-751. Recurrent selection will be used to increase oil and protein content, reduce mucilage content and improve agronomic performance within this population. Recurrent selection within an oilseed quality population is a strategic methodological shift from backcrossing which involves condiment types. This shift is aimed at eliminating delays and reducing the workload associated with re-selecting oilseed quality plants among progeny of oilseed quality x condiment *S. alba* crosses. Concurrent selections will be employed to reduce total glucosinolate content, increase oleic acid content and reduce linolenic acid content , in addition to recurrent selection for increased oil and protein content, as well as agronomic improvement.

2. *Increasing oil content in oilseed Sinapis alba*

Significant increases in oil content must be achieved before *S. alba* will become a competitive oilseed species for production in Canada. Oil content is a quantitative trait and can be effectively improved utilizing recurrent selection (Olsson, 1974). This approach, however is slow, and approaches such as backcrossing to high oil content (36 -38%) germplasm from Sweden or intergeneric crosses with high oil content (48 - 50%) *Brassica* species may be more efficient. Oil content in condiment *S. alba* is low (30%) and it is ultimately desirable to increase this content by 10-15%. This process has been successfully initiated in this research. Oil content is a quantitative trait requiring a recurrent procedure of introgressing and accumulating oil content genes. In this research oilseed strains of *S. alba* were backcrossed to the Swedish high oil content parent (Svalöf) three times. Resultant oilseed progeny from this procedure were evaluated in the field in replicated trials in 1997, 1999 and 2000. All the lines evaluated confirmed that oil content of this material has increased by an average of 3.5% (Table 3). Oilseed-quality lines with the highest oil content (35.9 to 36.8%) were utilized in the breeding crosses to develop the broad based oilseed *S. alba* population.

3. *Evaluating Sinapis alba germplasm accessions*

An important activity in this research was the evaluation of adapted and introduced *S. alba* germplasm, with the goal of identifying accessions of value to the oilseed *S. alba* breeding program. This research was focused on evaluating variation for the purpose of expanding the germplasm base. Broad genetic diversity is essential in breeding populations, to ensure long-term potential for genetic gain and to mitigate against genetic vulnerability to biotic and abiotic stresses. This consideration is particularly important in this program, where the foundation of the oilseed *S. alba* is being developed. Expanding the

genetic diversity in the oilseed *S. alba* program requires the use of both locally adapted condiment mustard varieties as well as introduced accessions.

One hundred and thirty-five introduced accessions of *S. alba* were evaluated in the field for agronomic performance and seed quality in 1997, 1998 and 1999. The accessions were derived from collections in various gene banks and research institutions in Europe and represent a wide diversity of *S. alba*. Adapted cultivars such as AC Base, AC Pennant, and Viscount were utilized as experimental checks together with a high oil content breeding line, Svalöf The experiments were established at the Saskatoon Research Centre field station in early to mid May, depending on spring conditions, in an non-replicated nursery in 1997, and in replicated trials (Randomized Complete Block Design) with three replications in 1998 and four replications in 1999. Plots were assessed for seedling emergence, seedling and mature plant vigour, number of days to flowering (DTF), plant height at maturity, lodging, number of days to maturity (DTM), seed yield and agronomic performance. Harvested seed was analysed for green seed, seed weight, oil, protein and mucilage content, fatty acid and glucosinolate profiles.

The results (Table 4) indicated that most accessions exhibited agronomic traits similar to the check varieties AC Base, and Viscount, averaging 39 days to flowering (DTF) and 89 days to maturity (DTM). Plant height of the accessions was on average taller (138 cm) then AC Base (109 cm) and concurrently, most germplasm accessions were more vegetative in growth habit than AC Base. There were some notable variants for these agronomic traits. Three accessions were earlier (33-35 DTF, and 74 DTM) and shorter (less than 120 cm) than the other experimental lines. These lines were also very uniform, with a less vegetative growth habit. A very late maturing line (50 DTF and 102 DTM) was also identified. This line was also short, uniform and had a distinct morpho-type, with relatively little vegetative growth.

Yield was measured in 1999, in a multi-location trial conducted in Saskatoon (two planting dates) and Scott. This trial consisted of three 25 entry tests at each of three locations, arranged in a 5x5 lattice design, with 4 row plots, and four replications per plot. The checks were AC Base (well adapted, high yielding) and Svalöf (high oil content). AC Base yielded 2449, 2879 and 2285 kg/ha at Scott, Saskatoon May seeding and Saskatoon June seeding, respectively. Germplasm accessions on average yielded 80-95% of AC Base. Svalöf, at 101 days to maturity (DTM) was 5 days later maturing than AC Base. Most germplasm accessions matured between 95 and 101 DTM with one notable late (106 DTM) and three early (80 DTM) maturing exceptions. On average germplasm plant height at maturity was similar to AC Base (123 cm) with some notable tall exceptions (133 - 151 cm). Svalöf was 5 - 6 cm taller than AC Base at all locations. Average germplasm seed weight (5.6 g 1000^{-1} seed) was on average smaller than that of AC Base (5.8 g 1000^{-1} seed). No useful variants were detected among the germplasm accessions for oil or protein content. Average germplasm oil content was comparable to AC Base (30.5%) which was in turn significantly less than the oil content in Svalöf (37.6%). Similarly germplasm seed protein content was comparable to AC Base (32.1%) and higher than that of Svalöf (27.0%). Similarly the fatty acid profiles of the germplasm accessions were comparable to adapted condiment yellow mustard (Table 5). One accession was observed to possess pods which occasionally carried an additional seed in the beak of the pod. This trait has been reported in some wild forms of *S. alba*. Plant structure and pod morphology

observations are important when evaluating accessions, as these characteristics may play an important role in breeding for increased seed yield by modifying plant form and harvest index. There are relatively few reports in the literature of *S. alba* germplasm evaluation (Yaniv, 1994; 1995a; 1995b) and this research will be a valuable contribution to that information base.

4. *Increasing protein content in Sinapis alba*

The second project objective requires the development of populations with high protein content. Adapted condiment mustard lines are a valuable resource for this breeding program. Recurrent selection is an effective strategy for increasing the content of a quantitative trait such as protein. Protein content had not previously been selected for in the experimental populations and response to selection was evident. *Sinapis alba* seed coat contains a high content of mucilage (Cui *et al.*, 1995a; 1995b). High mucilage content in the meal inhibits animal feed intake and reduces digestibility, especially in non-ruminant livestock. It is therefore important to select for reduced mucilage content in oilseed *S. alba* to mitigate against this potential barrier to market entry.

Selection for increased protein content was initiated using elite condiment yellow mustard germplasm from the Saskatoon Research Centre. In 1997, one thousand and ninety-two half-sib lines were evaluated for agronomic performance and seed quality in a two replicate field evaluation nursery, in a 9 x 9 lattice block design. The high protein check was AC Pennant, a well adapted condiment yellow mustard cultivar. In the field, plots were evaluated for agronomic performance, and vigour. Seed samples were taken from each plot for the determination of oil (NMR method), protein (Dumars combustion) and mucilage (viscosity measurement). Selection was based on maximum oil content, and reduced mucilage content. Twenty-six half-sib lines were selected, which combined high protein content (>38% on whole seed) and reduced mucilage (less than the average population viscosity, 37 cS/gm sd (flow time of water/seed wt/water wt)) (Table 6)

A crossing block was established in the field in 1998, consisting of 2600 plants (100 plants from each of the selected high protein content lines) to initiate another cycle of recurrent selection for increasing seed protein. One thousand individual plants were harvested from the crossing block for half-sib evaluation in 1999. In addition replicated yield trials were conducted in 1998 to determine agronomic and seed quality characteristics of the 26 advanced high protein breeding lines. Agronomic rating of the lines was good to excellent, with similar height (135 cm), days to flowering (38) and days to maturity (79) to AC Base. Variation for agronomic traits was low in this material.

In 1999, nine hundred and seventy-two half-sib lines of *S. alba*, derived from single plants harvested from the 1998 high protein crossing block, were evaluated in a 9 x 9 lattice block design with 12 tests, two row plots and two replications. Check lines were AC Base, Svalöf, and a seed bulk of the 1998 high protein crossing block. Agronomic rating of these lines is good to excellent and comparable to AC Base. The lines were evaluated for protein and mucilage content. The selection criteria was a two step

culling process. First, 100 lines were identified with protein content of 1.5% or greater than the 1998 high protein population bulk. The high protein population bulk was a more stringent check for protein content because it had consistently higher protein content than AC Base (an average 1.66% higher). The second selection criteria was mucilage content and thirty plants with mucilage content (32.3 Centistokes) less than AC Base (39.3 Centistokes) were then selected for the 2000 crossing block. The average mucilage content of selected lines was 32.3 Centistokes compared to 39.3 Centistokes in AC Base. The range in protein and mucilage content among selected lines (46-51% meal protein; 23-39 Centistokes for mucilage) indicates that continued response to selection for these traits is possible.

In 2000, a crossingblock was established in the field from remnant seed of 30 plants selected for high protein content and reduced mucilage content, based on the 1999 half-sib evaluation nursery. One hundred seeds from each of the 30 selected lines were planted. This population is well adapted to western Canada, and has the same genetic background as condiment yellow mustard varieties AC Pennant and AC Base. Lines selected from this recurrent selection procedure are important parental sources for high protein content, reduced mucilage content and good agronomic adaptation in oilseed *S. alba* breeding. Recurrent selection continued with a half-sib evaluation nursery of nine hundred and seventy-two half-sib lines in 2001.

5. *Inheritance of erucic acid and hydroxybenzyl glucosinolate in Sinapis alba.*

Understanding the genetic control of important traits is critical for developing efficient breeding strategies. Genetic factors influence decisions on the choice of breeding methodology, timing of trait selection in the breeding process, population size, and optimum trait combinations in simultaneous or sequential selection procedures. In the oilseed *S. alba* breeding program the most critical crop defining traits that enable the initial development of the crop are low erucic acid content in the oil and low glucosinolate content in the meal.

An important goal of this study was to determine the inheritance of erucic acid and hydroxylbenzyl glucosinolate (the main glucosinolate of *S. alba*). Research on the development of edible oilseed *S. alba* and associated genetic studies have been reported from Idaho (Tang *et al.*, 1996). These authors reported that total glucosinolate content is controlled by the maternal plant genotype, and inheritance is in a simple Mendelian manner, with the low glucosinolate characteristic being recessive to high glucosinolate levels. This mode of inheritance is consistent with that reported for *Brassica*. For this type of inheritance, backcrossing (with a test cross to identify heterozygotes) is an effective breeding method to introgress the low glucosinolate characteristic into high glucosinolate adapted germplasm (Downey and Rakow, 1987).

Genetic control of fatty acid composition in seed oil of *S. alba* is reported to be under additive gene action with partial dominance for the reducing alleles for oleic and linoleic acids and partial dominance for the enhancing alleles for erucic acid (Ecker and Yaniv, 1993; Tang, 1996). Erucic acid is strongly negatively correlated with oleic acid, suggesting a genetic interdependence between these two fatty acids.

Broad sense and narrow sense heritability estimates for each of oleic acid, linoleic acid and erucic acid were high in both citations.

In this project the research on inheritance of erucic acid and hydroxybenzyl glucosinolate was conducted using classical genetic analysis, by a graduate student with partial support from the Saskatchewan Canola Development Commission. In 1997 much of the work toward attaining this goal entailed making crosses to develop F1, BC1F1, and F2 generations of specific crosses involving parents with contrasting profiles in erucic acid and glucosinolate content. Sabre has high erucic acid content (55%), AC Pennant has medium erucic content (35%), while WD96-1 (2%) and WD96-2 (1%) have low erucic acid content. In addition Sabre and AC Pennant have high glucosinolate content while WD961 and WD96-2 have low glucosinolate content. Leaf tissue was taken and freeze dries from all parents and their progeny for the development of molecular markers for erucic acid.

In 1998 activities under this goal were focussed on analysing laboratory data from F1, BC1F1, and F2 generations of crosses made in 1997. Chi square analysis of progeny segregation patterns indicated that erucic acid content in *S. alba* is determined by the genotype of the embryo, and controlled by a single gene, exhibiting partial dominance of high over low erucic acid. In 1999 the genetic analysis of hydroxybenzyl glucosinolate concluded that this trait is a single gene trait, under maternal control, with dominance of high over low glucosinolate content. Molecular markers for erucic acid were developed in 1999, and will be valuable in identifying future low erucic acid content lines in the oilseed *S. alba* breeding program.

6. *Screening Sinapis alba germplasm accessions for resistance to white rust*

Sinapis alba does not exhibit susceptibility to white rust in the field and in laboratory screenings, it is rated as moderately resistant. One hundred and eighty introduced and adapted accessions and cultivars of *S. alba* were screened for resistance to white rust races under controlled environmental conditions in a growth chamber. This work was conducted in collaboration with Dr. Lorne Duczeck and Dr. Roger Rimmer. The *S. alba* accessions were screened for reaction to white rust, races 2A, 2V, 7A and 7V. Most *S. alba* lines were rated as moderately resistant with a few lines rated as resistant or susceptible. Field evaluations of all lines indicated that an aggressive breeding program for white rust resistance is not important in *S. alba*. However, it will be important to screen breeding lines to ensure that susceptibility to this pathogen is not introduced through the breeding process.

7. *Screening oilseed Sinapis alba lines for resistance to blackleg.*

Blackleg is an important disease of canola (Gugel and Petrie, 1992). *Sinapis alba* is resistant to this disease and the importance of screening breeding material is to ensure that selection activities during the breeding of oilseed *S. alba* do not inadvertently introduce susceptibility for blackleg. This screening was conducted in collaboration with Mr. Richard Gugel, a pathologist at the Saskatoon Research Centre. In 2000, twenty-four oilseed *S. alba* breeding lines (representing the full range of genetic material for oilseed *S. alba)* and two condiment *S. alba* cultivars were screened for blackleg resistance. The *B. napus* cultivar

Westar was used as the susceptible check. All *S. alba* entries were highly resistant to blackleg, with ratings of 0.0 - 0.12 (0=healthy, 5=dead) while the susceptible check was rated at 1.8 - 3.08. Thus modifications to eliminate erucic acid, hydroxybenzyl and benzyl glucosinolate content in oilseed *S. alba* have not resulted in increased susceptibility to blackleg infection. Ongoing disease and pest monitoring of oilseed *S. alba* breeding lines is important to ensure that resistance factors currently present in the condiment germplasm are not eradicated during the process of breeding oilseed strains.

Results and conclusions

The *S. alba* oilseed breeding program had successfully stabilized three important oil and meal quality traits: <0.1% erucic acid, <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate content. This research has been successful in eliminating the major glucosinolate in condiment *S. alba*, hydroxybenzyl glucosinolate, and one of the minor glucosinolates, benzyl glucosinolate. There are however elevated level of other minor glucosinolates, in particular, hydroxy-butenyl and indolyl glucosinolates which require further breeding in order to be eliminated. The fatty acid profile of oilseed *S. alba* is excellent, with variation for very high oleic acid content (>75%) and low linolenic acid content (<4%). Such improvements in fatty acid composition will enable this vegetable oil to be strategically marketed as a competitive, high quality product.

The development of a broad based oilseed-quality population was an important milestone in the oilseed *S. alba* breeding program. This population constitutes the founder population for the new oilseed *S. alba* crop. All plants in this population contain <0.1% erucic acid, <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate content. The population has also been introgressed with genes for high oil and low mucilage content from the breeding line Svalöf, as well as good agronomic performance from AC Base, and high protein content form SA97-751. This result is significant and demonstrates that successful development of oilseed *S. alba* is possible at the Saskatoon Research Centre. There is a strong justification for the continued support of this research when one considers these advances, together with the tremendous potential that this crop offers to farmers, oil and meal processors in the semi-arid prairies, and to the Canadian oilseed industry as a whole.

Oil content has been increased by an average of 3.5%. The oilseed *S. alba* population has been introgressed with genes for high oil content and further increases in oil content can be expected through recurrent selection. Significant increases in oil content must be achieved before *S. alba* will become a competitive oilseed species for production in Canada. Oil content is a quantitative trait and can be effectively improved utilizing recurrent selection (Olsson, 1974). This process is time-consuming but effective.

The Saskatoon Research Centre has one of the largest collection of *S. alba* accessions in the world. These were evaluated in this project and provide valuable variability for ensuring that the genetic base of the breeding program is sufficiently broad to enure continued response to selection and to mitigate

against genetic vulnerability to biotic and abiotic stresses. Results from the germplasm evaluation are valuable for further population and cultivar development.

Increasing oil content is an important goal for the high protein content oilseed *S. alba* development. Protein content in *S. alba* is already high at 48% of the defatted meal. Through this research protein content was increased steadily through successive cycles of recurrent selection at a rate of 1% per cycle. This high protein population is well adapted to western Canada, and has the same genetic background as the leading condiment yellow mustard varieties AC Pennant and AC Base. Lines selected from this recurrent selection procedure are important parental sources for high protein content, reduced mucilage content and good agronomic adaptation in oilseed *S. alba* breeding. A high oil content line SA97-751 was utilized as a parent in the development of the broad based oilseed *S. alba* population.

The genetic control of the two critical traits for developing oilseed *S. alba* were elucidated during this project. Erucic acid content in *S. alba* is determined by the genotype of the embryo, and controlled by a single gene, exhibiting partial dominance of high over low erucic acid content. Hydroxybenzyl glucosinolate is also a single gene trait. It is under maternal control, with dominance of high over low glucosinolate content. Molecular markers for erucic acid were also developed. This genetic information and molecular marker technology is valuable for making subsequent methodology decision as well as improving the efficiency of selection through marker assisted selection.

The disease and pest resistance profile of *S. alba*, is excellent and it is important that during the development of oilseed *S. alba* vulnerabilities are not introduced which would make the species susceptible to important pests and diseases. During this project germplasm was evaluated for reaction to blackleg and white rust. There is no evidence that the breeding program has introduced any susceptibility for these diseases into the oilseed *S. alba* germplasm.

The development of oilseed *S. alba* is a long-term strategic research program. This project has demonstrated that the development of this new oilseed crop is feasible and has made important contributions to the development of the basic foundational germplasm. Important results have also been attained which contribute to increasing the efficiency of subsequent strategies for continuing with this research.

Publications

W.J. Drost. 1999. Inheritance of canola-quality traits and identification of molecular markers for erucic acid content in *Sinapis alba* L. M.Sc. thesis. Department of Plant Sciences, University of Saskatchewan, Saskatoon.

F. Katepa-Mupondwa, G. Rakow, P. Raney and R. Gugel. 1999. *Sinapis alba*: A new oilseed crop for the prairies. Soils and Crops' 99 Conference Proceedings. University of Saskatchewan 364-366.

F. Katepa-Mupondwa, G. Rakow and P. Raney. 1999. Developing oilseed yellow mustard (*Sinapis alba* L.*)* in western Canada. Proceedings of 10th International Rapeseed Congress, Canberra, September 26 - 29.

F. Katepa-Mupondwa. 1999. Chemical composition of *Sinapis alba* L. germplasm. A report submitted to director, AAFC, Saskatoon Research Centre, based on data from research conducted by Drs. P. Raney and G. Rakow.

F. Katepa-Mupondwa, G. Rakow and P. Raney. 1999. Meal quality characteristics in yellow mustard (*Sinapis alba* L.*)*. Proceedings of 10th International Rapeseed Congress, Canberra, September 26 - 29.

Katepa-Mupondwa, F., G. Rakow and P. Raney. 2000. Development of oilseed *S. alba.* AFIF Conference, Regina, Saskatchewan, November 7-8.

Rakow, G., D. Potts, P. Raney and F. Katepa-Mupondwa. 2000. Designing oilseed crops for the Canadian dry prairie. 3rd International Crop Science Congress, Hamburg, Germany. Poster.

Publications currently in preparation

Katepa-Mupondwa, F., P. Raney, G. Rakow, and R. Gugel. Diversity analysis of *Sinapis alba* L. accessions for agronomic and seed quality traits.

Katepa-Mupondwa, F., P. Raney, G. Rakow, and R. Gugel. Germplasm description of high protein, low mucilage content *Sinapis alba* L.

Katepa-Mupondwa, F., P. Raney, and G. Rakow. A study of population variation in four cycles of recurrent selection for increased protein content in *Sinapis alba* L .

Indications for further development

This research is part of a long-term strategic crop development initiative for the semi-arid prairies. This project has laid the foundation for a strong breeding program which can successfully bring oilseed *S. alba* to market, for the benefit of western Canadian producers and the oilseed industry. The next major research challenges include reducing total glucosinolate content (<12 μmol/g seed), increasing oil content (>45%), increasing seed yield (to be competitive with grain crops in the semi-arid prairies), and reducing seed coat mucilage. In order to develop a premium-quality, highly competitive oil, research is needed to increase oleic acid (>80%) and reduce linolenic acid (<3%) content. There is indication that variability exists to reach these objectives. Variability still exists for continued increases in protein content. The Saskatoon Research Center has the germplasm, technical knowledge and facilities to develop this

technology. Continued funding of this project is important and will benefit many sectors of the oilseed industry.

PERSONNEL

Dr. Katepa-Mupondwa was hired as a scientist (oilseed breeder) in 1997 to coordinate oilseed *S. alba* breeding activities at the Saskatoon Research Centre. She conducted this project in collaboration with Dr. G. Rakow (Section Head, Crop Breeding and Diversity), Dr. P. Raney (oilseed chemist), Dr. J. Soroka (entomologist), Dr. R. Rimmer (pathologist) and Mr. R. Gugel (pathologist and germplasm curator). The chemical analyses (seed oil and protein content, fatty acid composition, glucosinolate content) were carried out by laboratory technicians. Technicians and assistants were also involved with field nursery work, growth chamber and greenhouse breeding activities, as well as disease resistance screening activities.

EQUIPMENT

Equipment for laboratory analysis and field nursery breeding activities was provided by the Saskatoon Research Centre, AAFC. A Near Infrared Reflectance Spectrophotometer (NIR) was purchased as a shared cost between this AFIF project and project 1a XVIII. The cost of the NIR to this project was $40, 000.

INNOVATION FUND ACKNOWLEDGEMENT

During the project period, numerous visitor groups including the Mustard Growers Association, the Plant Biotechnology Institute, the Canadian Seed Growers Association and researchers from various companies and institutions toured the oilseed *S. alba* research sites and support from AFIF was acknowledged. AFIF support was also acknowledged in oral presentations, posters and informal discussions at various local and international meetings including, the International Crucifer Genetics Workshops in Montreal (1998) and Davis, California (2002), the International Rapeseed Congress in Canberra (1999), the Soils and Crops Conference, University of Saskatoon (1999), The Governor General's Conference (2000), the Saskatoon Research Centre Advisory Committee Presentations (2000 & 2001) and at PRRCG meetings (1998-2002). AFIF was also acknowledged at various meetings with representatives from various industry and public research organizations. The *S. alba* project has a high profile at the Saskatoon Research Centre, and a separate study outline was developed in 1998, presented to AAFC management in Ottawa and approved. The study outline acknowledges AFIF support of the project.

PROJECT PUBLIC INFORMATION MATERIALS

EXPENSE STATEMENT

Attached

DELIVERABLES FOR EVALUATION

Ten milestones were identified in the grant proposal and have been met as indicated.

1. Field evaluate agronomic performance and seed quality of *S. alba* germplasm and compare its performance with that of *S. alba* condiment mustard varieties to identify the major weakness of this germplasm that need to be improved.

   Germplasm accessions represent valuable genetic diversity which enhance the oilseed breeding program. Germplasm accessions were on average not as well adapted as local condiment lines, however this adaptation can be improved through breeding. There are no other reported evaluations of such a large collection of *S. alba* germplasm in the literature, and information from this evaluation may be of value to other *S. alba* breeders. This milestone was met through activity #2.

2. Determine the genetic control (inheritance) of erucic acid and glucosinolate contents of oilseed *S. alba* and use this information to formulate efficient breeding strategies for the introgression of these seed quality traits into elite breeding populations.

   The genetic control of the two critical traits for developing oilseed *S. alba* were elucidated. Erucic acid content in *S. alba* is determined by the genotype of the embryo, and controlled by a single gene, exhibiting partial dominance of high over low erucic acid. Hydroxybenzyl glucosinolate is also a single gene trait. It is under maternal control, with dominance of high over low glucosinolate content. Molecular markers for erucic acid were also developed. Such qualitative inheritance patterns suggests that these traits are easily fixed in a population and can be fixed early in the breeding program. In addition, methodologies such as backcross breeding may be effective. Information pertaining to the degree of maternal control is critical for ensuring that trait evaluations are made in the correct generation of expression. The molecular markers for erucic acid are important tools for marker assisted selection of this trait. This milestone was met through activity #5

3. Increase seed oil content of oilseed *S. alba* through introgression of genes for high oil content from a European high oil content *S. alba* source by backcrossing. Three to five generations of backcrosses might be required, and stepwise increases in oil content are expected from each additional backcross generation.

Three backcrosses were made to the high oil content breeding line Svalöf, resulting in oilseed *S. alba* lines with an average oil content increase of 3.5%. A steady increase in oil content is expected with recurrent selection in the oilseed *S. alba* population. The goal to increase oil content remains critical for the successful establishment of oilseed *S. alba* as a crop. This milestone was met in activity #2.

4. Increase meal protein content of *S. alba* to above 50% on a dry, oil free meal basis through standard recurrent selection strategies on an ongoing basis through the duration of the project for four years; and begin introgression of the zero erucic acid and low glucosinolate traits into high protein germplasm during the last year of the project.

Three cycles of recurrent selection have resulted in *S. alba* lines with protein content above 50% (meal basis). Variability exists for continued response to selection for this trait in this population. The high protein content population is a valuable source of parents for the high protein content oilseed *S. alba* program. In fact, a high protein selection (SA97-751) was utilized as a parent in the development of the broad based oilseed *S. alba* population. Increasing protein content was conducted under activity #4.

5. Identify *S. alba* plants that are basically free of benzyl and hydroxybenzyl glucosinolate through single plant selections from the oilseed quality germplasm base.

In the first two years of this project the most significant milestone was the development of oilseed-quality lines which combined the traits for <0.1% erucic acid, <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate content. Low content of erucic acid in the oil and glucosinolates in the meal are crop defining traits that enable the development of an edible oilseed crop in Brassicas. Another important achievement was the development of the first broad based oilseed *S. alba* population, which constitutes the foundation of the future oilseed *S. alba* crop. This achievement has put the concept of oilseed *S. alba* beyond the proof of concept phase. With sufficient funding and time, the Saskatoon Research Centre has the personnel, technical capacity and institutional framework to develop this new crop for western Canada. These achievements are described under activity #1.

6. Initiate selections aimed at decreasing linolenic acid content and simultaneously increasing oleic acid.

Oilseed-quality *S. alba* selections which met the requirement for <0.1% erucic acid, <0.1 μmoles g^{-1} seed hydroxybenzyl glucosinolate and <0.1 μmoles g^{-1} seed benzyl glucosinolate content, were also evaluated for other fatty acids especially, oleic, linoleic and linolenic acid. Low erucic acid content *S. alba* has increased oleic acid content ranging from 65 to 75% compared to 35% found

in condiment *S. alba*. Furthermore the variation observed for oleic acid suggests that further increase of this fatty acid is possible using conventional selection within the oilseed *S. alba* population. Fatty acid modification was conducted under activity #1.

7. Improve level of resistance to white rust through mass selection of resistant plants utilizing recurrent selection procedures. An initial screening of germplasm used for the research will be conducted in year one to establish levels of resistance to white rust races. Continued screening of the breeding material in years two three and four will ensure that high levels of white rust resistance are retained in the various breeding populations.

   Screening for reaction to white rust indicated that this disease is not serious in *S. alba*. It is however important to ensure that breeding material continues to be screened for white rust to ensure that high levels of resistance to white rust are maintained as oilseed *S. alba* is developed. White rust screening was conducted under activity #6.

8. Maintain high levels of blackleg resistance present in *S. alba* through annual evaluation of the breeding material in the AAFC-Saskatoon blackleg field disease nursery.

   Sinapis alba is highly resistant to blackleg, an important disease of canola. Screening of oilseed *S. alba* lines confirmed that these breeding lines are highly resistant and the breeding program has not introduced any vulnerabilities to this disease. Continued screening is important, to ensure that high levels of resistance to blackleg are maintained in oilseed *S. alba*. Blackleg screening was undertaken in activity #7.

9. Conduct multi-location field experiments to determine yield and seed quality (oil and protein content, fatty acid composition, and glucosinolate content) of oilseed *S. alba* developed from this research; and compare its performance with that of the starting material and condiment *S. alba* varieties to determine if the material warrants entry into co-operative oilseed variety registration tests.

   Field evaluations of oilseed *S. alba* breeding lines wre conducted in 1997, 1999 and 2000. These trials were at one location and confirmed that oilseed *S. alba* had increased oil content. The agronomic rating of these lines was below adapted check cultivars, which was not unexpected. In breeding, when selection is focussed on a few traits (in this case erucic acid and glucosinolates), there is some reduction in agronomic performance and such performance is recovered in subsequent breeding practices. Selection for improved agronomic performance is an important goal in the next phase of oilseed *S. alba* crop development, using the broad based oilseed *S. alba* population. Field evaluations of oilseed *S. alba* was conducted under activity #1.

10. Publish results of the research in peer reviewed scientific journals at the end of the project, and prepare collaborative research and development projects with private industry to ensure rapid commercialization of this new technology by the oilseed industry.

This project has resulted in the development of the basic germplasm which forms the foundation for developing oilseed *S. alba* as a new crop for the semi-arid prairies. Efforts are underway to develop research proposals for securing grants to address the next phase of developing this crop. Specifically such research will improve agronomic performance, increase oil content and develop lines that would be used in the developing of commercial cultivars by interested public and private institutions.

The following scientific manuscript are currently in preparation:

Katepa-Mupondwa, F., P. Raney, G. Rakow, and R. Gugel. Diversity analysis of *Sinapis alba* L. accessions for agronomic and seed quality traits.

Katepa-Mupondwa, F., P. Raney, G. Rakow, and R. Gugel. Germplasm description of high protein, low mucilage content *Sinapis alba* L.

Katepa-Mupondwa, F., P. Raney, and G. Rakow. A study of population variation in four cycles of recurrent selection for increased protein content in *Sinapis alba* L

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Table 1. **Fatty acid content* (%) of oilseed-quality experimental *Sinapis alba*, a Swedish high oil line and the cultivar AC Pennant.**

Sinapis alba	Oleic Acid C18:1	Linoleic Acid C18:2	Linolenic Acid C18:3	Erucic Acid C22:1
Oilseed	75.1	8.1	7.5	0.1
Svalof high oil	30	7.8	7.4	34
AC Pennant	28.8	8.2	9.5	32.7

* Fatty acid content based on greenhouse produced seed of 43 selected canola-quality plants, and on field produced seed of the Svalof high oil line and AC Pennant.

Table 2. **Content* of hydroxybenzyl, benzyl, and total glucosinolates (µmole/g seed) of oilseed-quality experimental *Sinapis alba* (mean of 43 Plants), a Swedish high oil line and the cultivar AC Pennant.**

Sinapis alba	Hydroxy-benzyl	Benzyl	Total Glucosinolate
Oilseed	0	5.1	6.3
Svalof Hi Oil	130.3	0.1	134.9
AC Pennant	155.6	0.1	160.4

* glucosinolate content based on greenhouse produced seed of 43 selected canola-quality plants, and on field produced seed of the Svalof high oil line and AC Pennant.

Table 3. **Oil and protein content of oilseed quality *Sinapis alba*, AC Pennant and a Swedish high oil content line.**

Sinapis alba	Oil (%seed)	Protein (%meal)
Oilseed	33.7	45.6
Svalof high oil	37.2	43.9
AC Pennant	29.5	45.7

Table 4. **Agronomic and seed quality traits of *Sinapis alba* germplasm accessions from field trials in 1997 & 1998.**

	Germplasm accessions 1997	Germplasm accessions 1998	AC Base
Days to flower	39	40	45
Plant height (cm)	102	138	109
Days to maturity	90	80	91
Seed weight (1000 sd)	5.6	5.6	5.4
Oil (% seed)	25.6	27.0	28.1
Protein (% meal)	49.2	49.2	47.0
Hydroxybenzyl glucosinolate (μmoles g^{-1} seed)	119	178	142

Table 5. **Fatty acid profile of germplasm accessions from field trials average of 1997 & 1998.**

	Germplasm 1997	Germplasm 1998	AC Base 1998
Fatty Acid			
Oleic	22.6	25.9	26.1
Linoleic	9.2	9.7	9.3
Linolenic	10.4	8.2	9.1
Erucic	39.2	35.6	33.4

Table 6 **Protein content of 26 selected high protein experimental lines compared to AC Pennant and a base population from which lines were selected**

Sinapis alba	Protein (%meal)	Seed weight (g/1000 seed)	Oil (%seed)
High protein lines	52.8	6.6	26.1
Base population	49.0	6.6	27.2
AC Pennant	47.6	5.7	28.3